Geomicrobiologcal characterization of Fe(II)- and Mn(II)-oxidizing activity at two mine drainage treatment sites

John M. Senko

Center for Environmental Chemistry and Geochemistry

and

Department of Civil and Environmental Engineering

The Pennsylvania State University

Mine drainage problems

- Acidity
- Sulfate
- Al
- Fe(II)
- Mn(II)

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Interested in the role of microbial activity in the (relatively) passive treatment of mine water, and how we can enhance Fe(II)- and Mn(II)-oxidizing activity for the removal of these mine-drainage constituents

Study sites

- **Glenwhite**
  - Mine discharge stream that empties into vertical-flow treatment system
  - Near Altoona, PA
- **Fairview**
  - Discharge from anoxic limestone drain
  - Flows through a shallow limestone bed
  - Elk County, PA

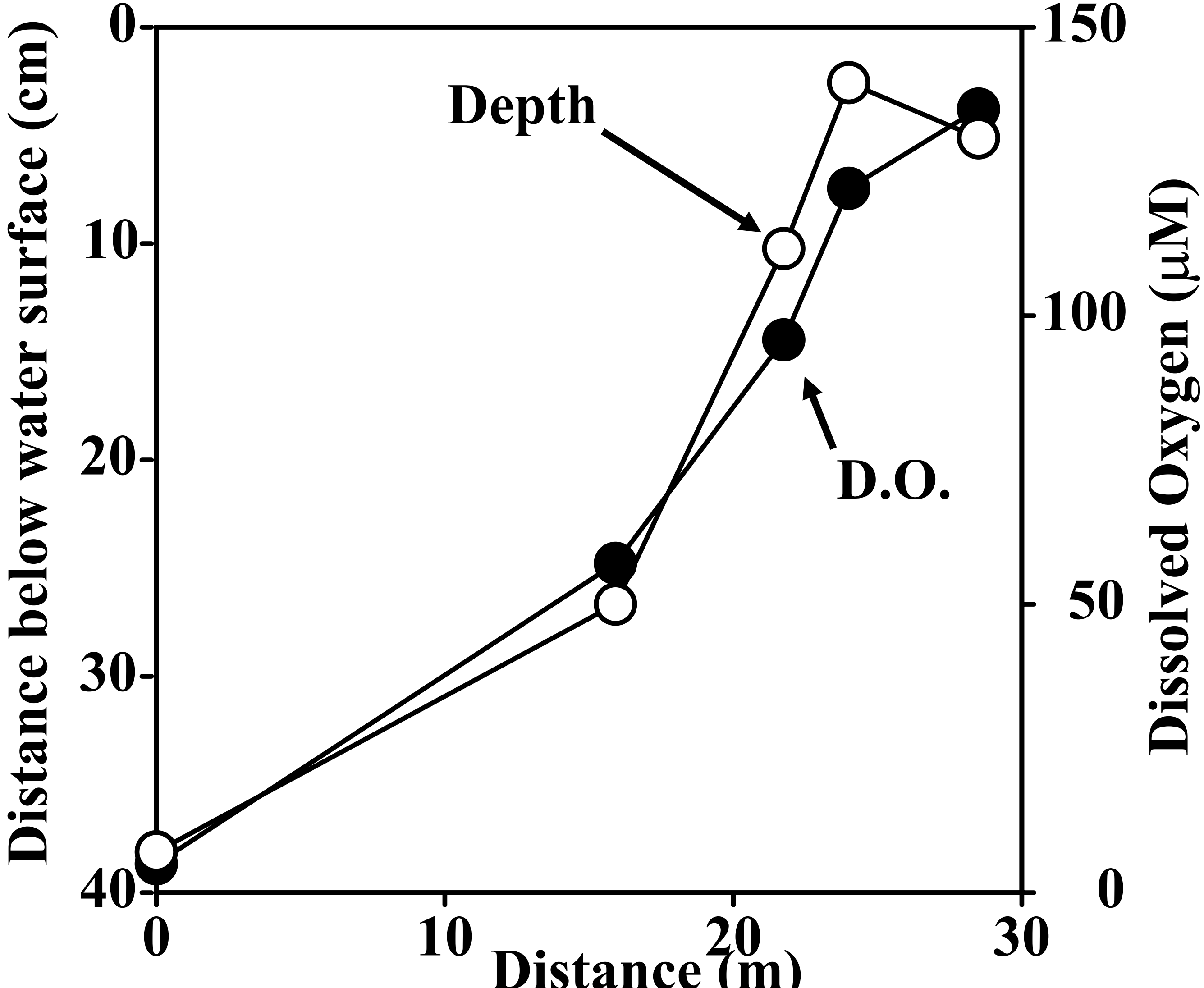
Glenwhite

Glenwhite discharge stream

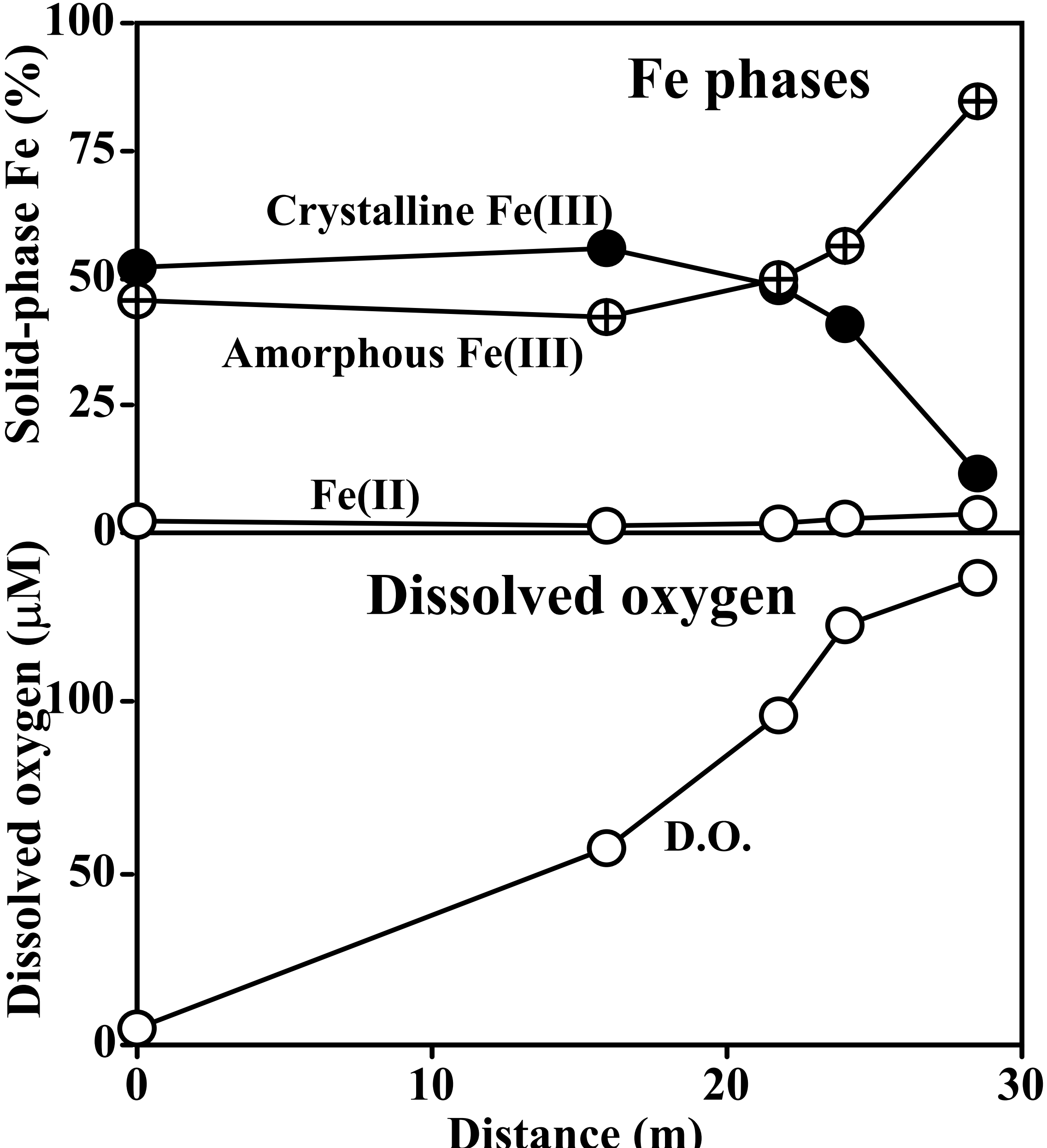
pH - 3.0 - 3.5
SO_4^{2-} - 3.4 mM
$Fe(II)_{sol}$ - 2 mM
$Fe(III)_{sol}$ - 40 μM

Mineral crusts recovered from drainage stream

Dissolved oxygen and water depth in Glenwhite discharge stream



Crystallinity of Fe phases in Glenwhite crusts

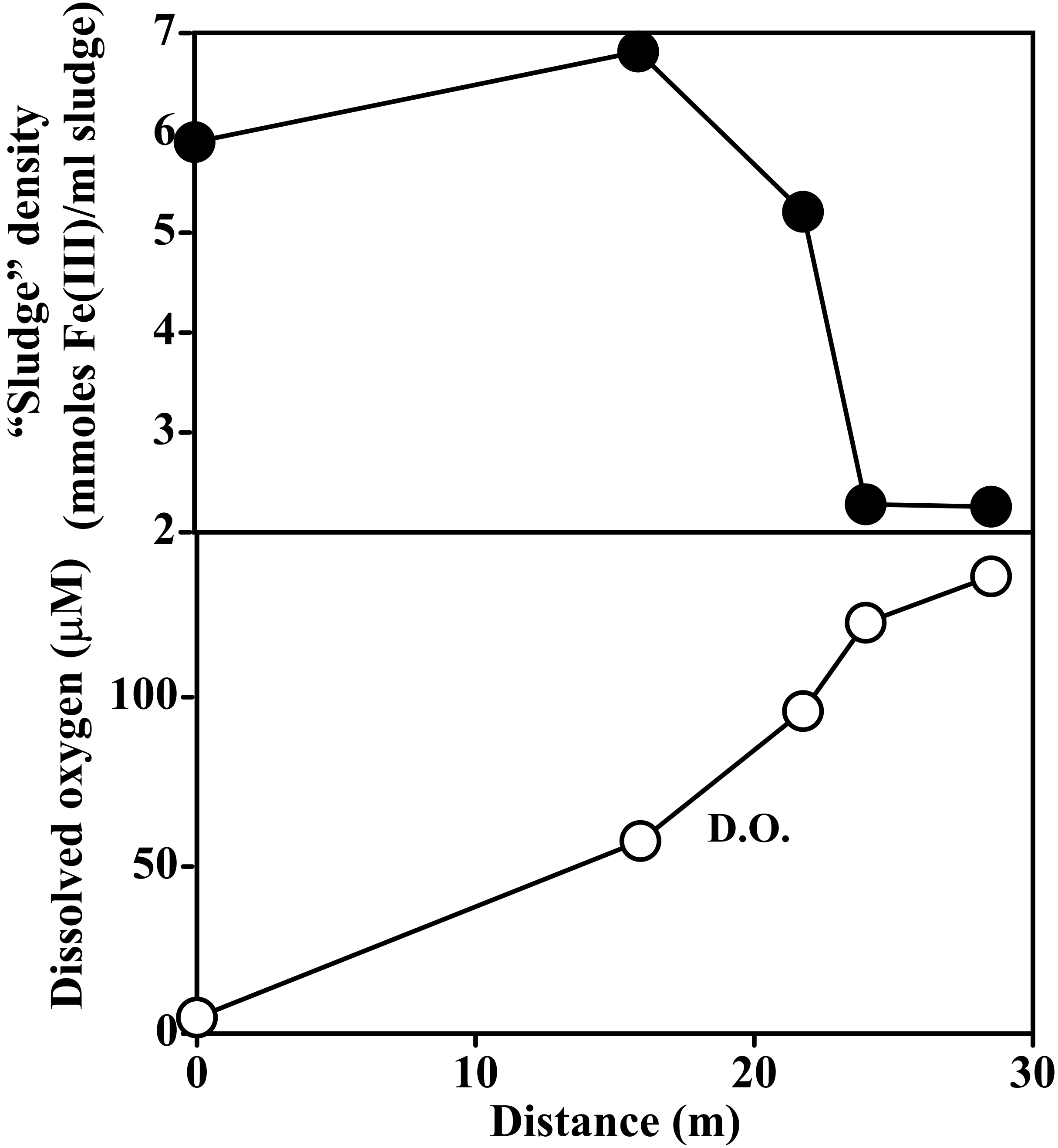


Fe(II) - 0.5 M HCl-extractable Fe

Amorphous Fe(III) - Hydroxylamine-HCl-extractable Fe

Crystalline Fe(III) - Dithionite-citrate-bicarbonate-extractable Fe

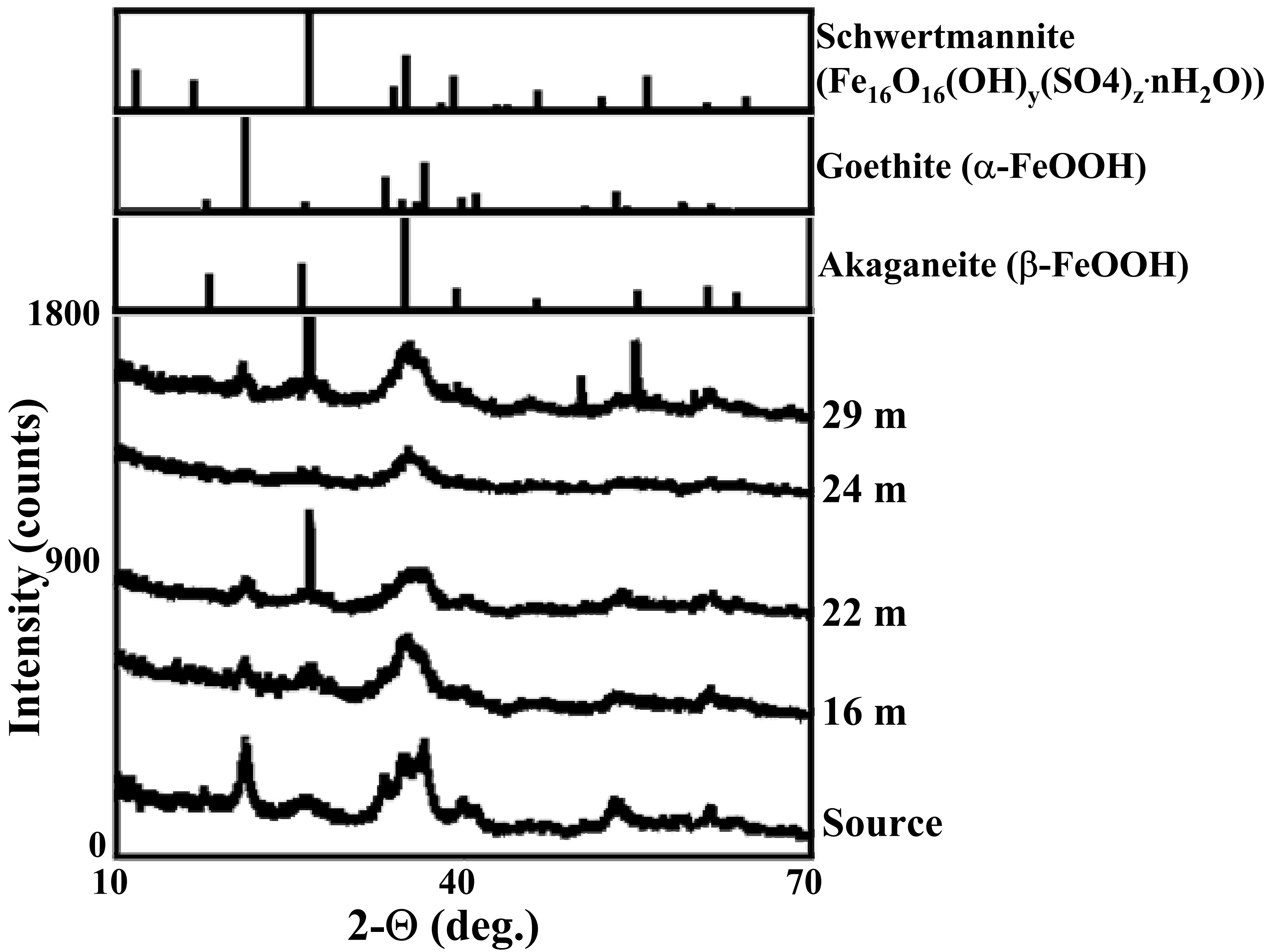
Density of Fe(III) crusts



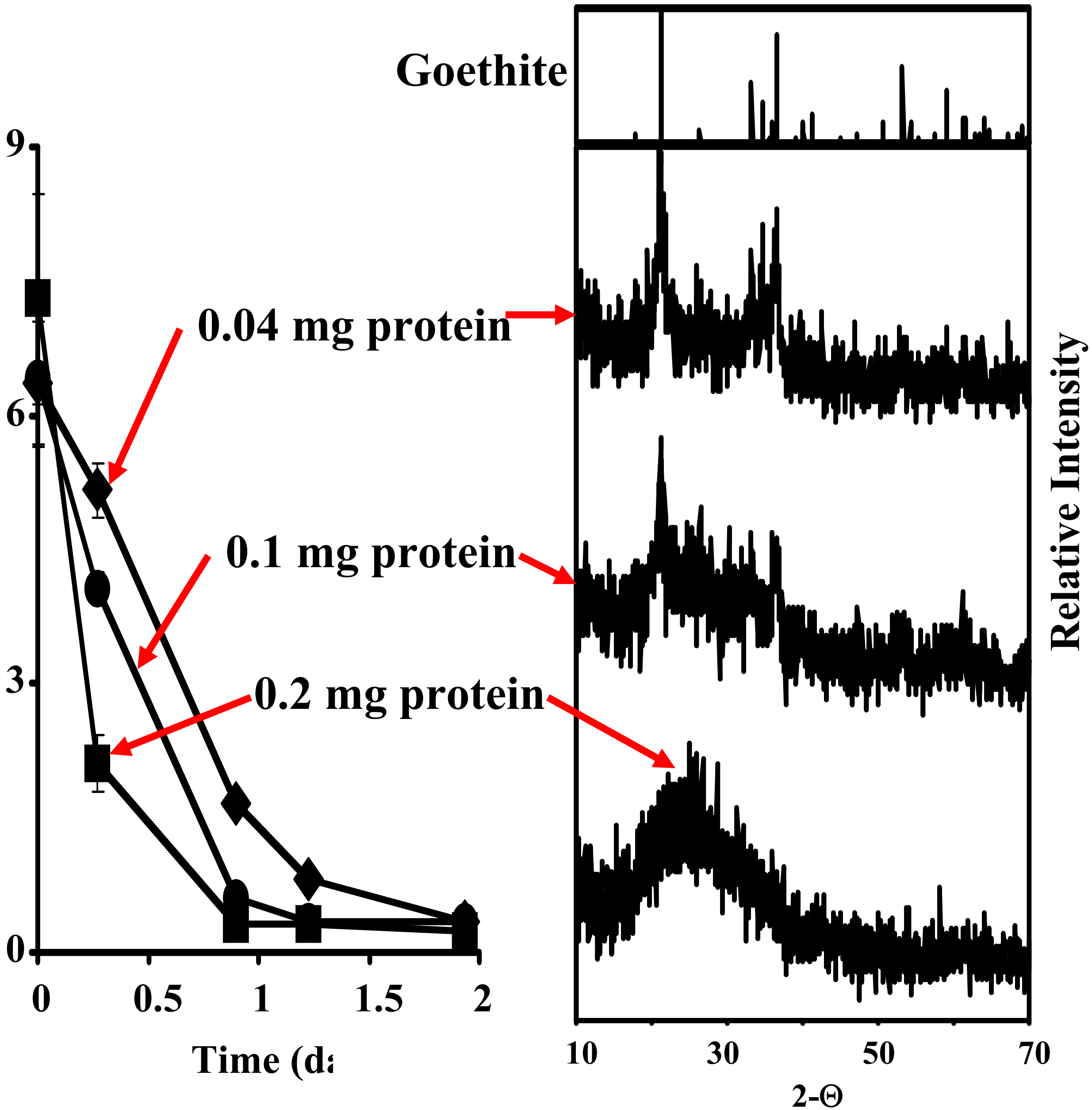
Fe(III)
"sludge density"

Dissolved
oxygen

X-ray diffraction analysis of Fe(III) crusts in Glenwhite discharge stream



The effect of Fe(II) oxidation rate on neutrophilic, nitrate-dependent, biogenic Fe(III) mineralogy

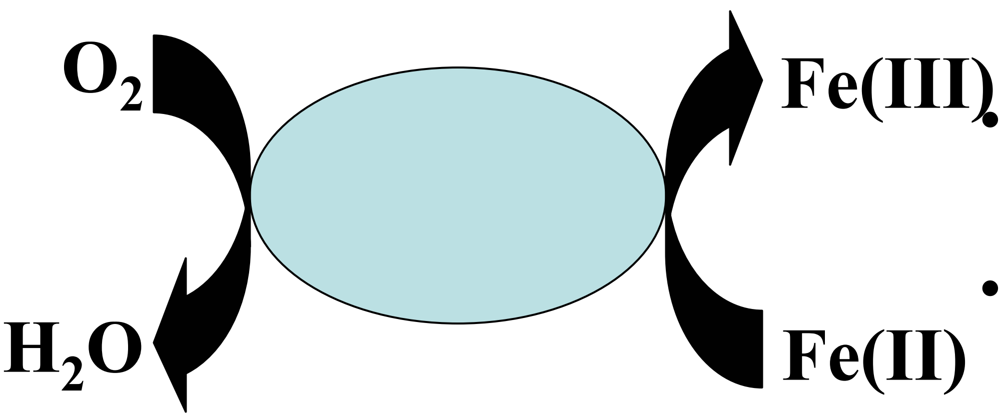


Fe(II)	Amorph. Fe(III)	Crystal. Fe(III)
5%	32%	63%
4%	50%	46%
4%	95%	1%

The -trophy case

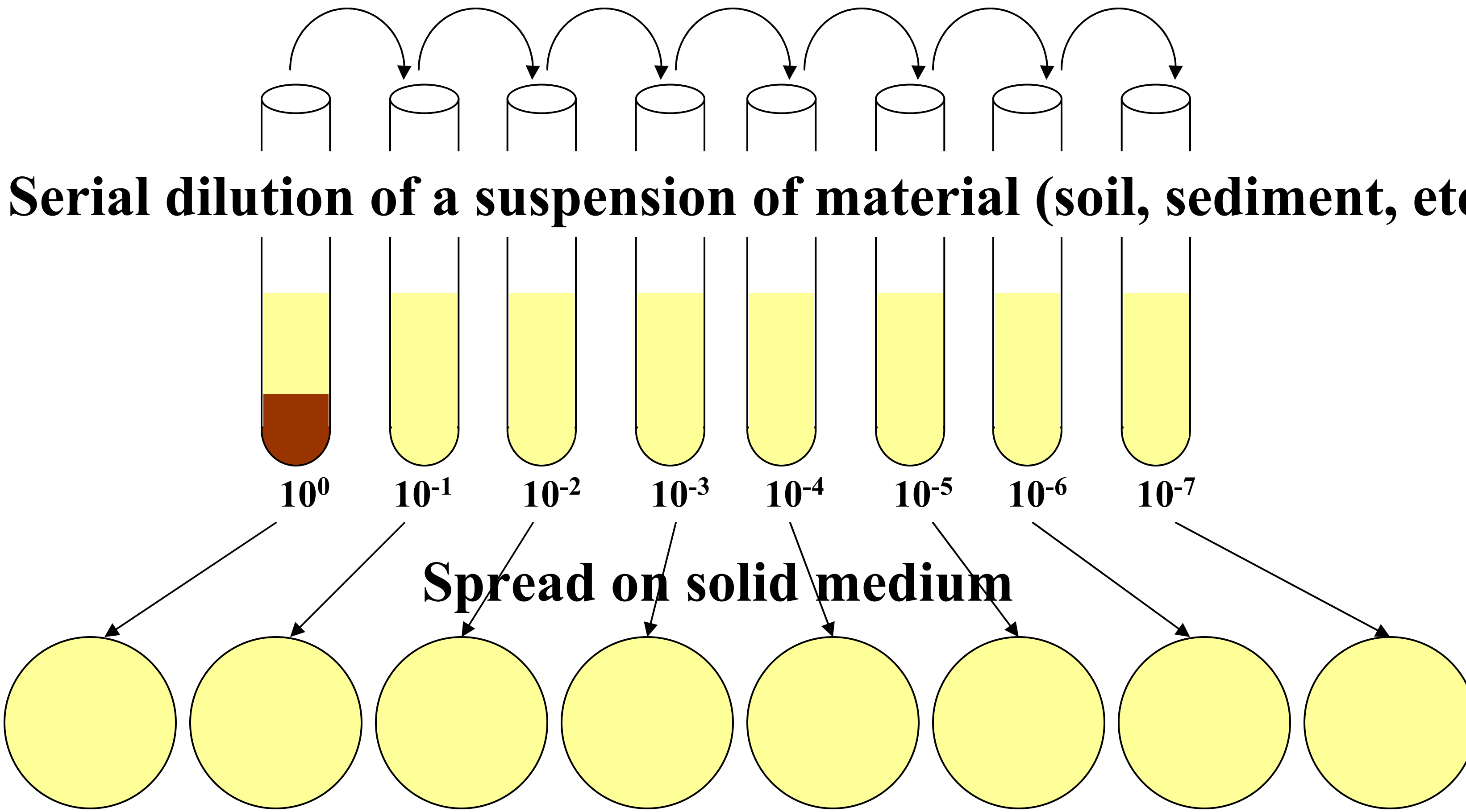
- **Chemo- vs. photo-trophy**
  - **Where the energy comes from: chemical or solar energy**
- **Litho- vs. organo-trophy**
  - **Where the electrons come from: inorganic or organic compounds**
- **Auto- vs. hetero-trophy**
  - **Where the cellular carbon comes from: fix CO_2 or assimilate fixed carbon**
- **I am a chemoorganoheterotroph.**
- ***Acidothiobacillus ferrooxidans* is a chemolithoautotroph**

Aerobic Fe(II) oxidation



- **Energy from Fe(II) oxidation coupled to O_2 reduction exploited for growth**
- **Lithotrophic, many are lithoautotrophic**
- ***Acidothiobacillus ferrooxidans, Leptospirillum ferrooxidans, Ferrimicrobium acidiphilium, Ferroplasma acidiphilium***

Enumeration of microorganisms



Count number of colonies formed

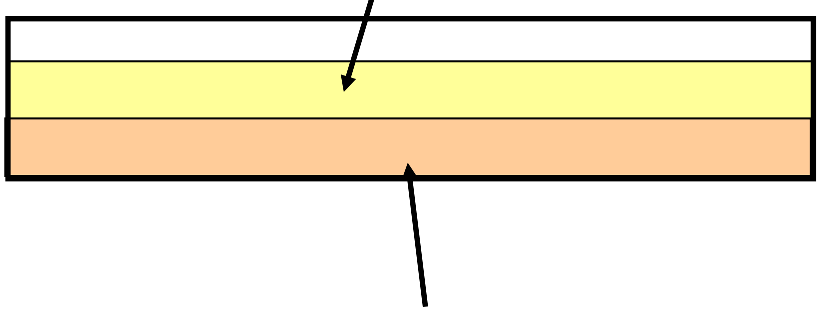
Assume 1 CFU = 1 cell

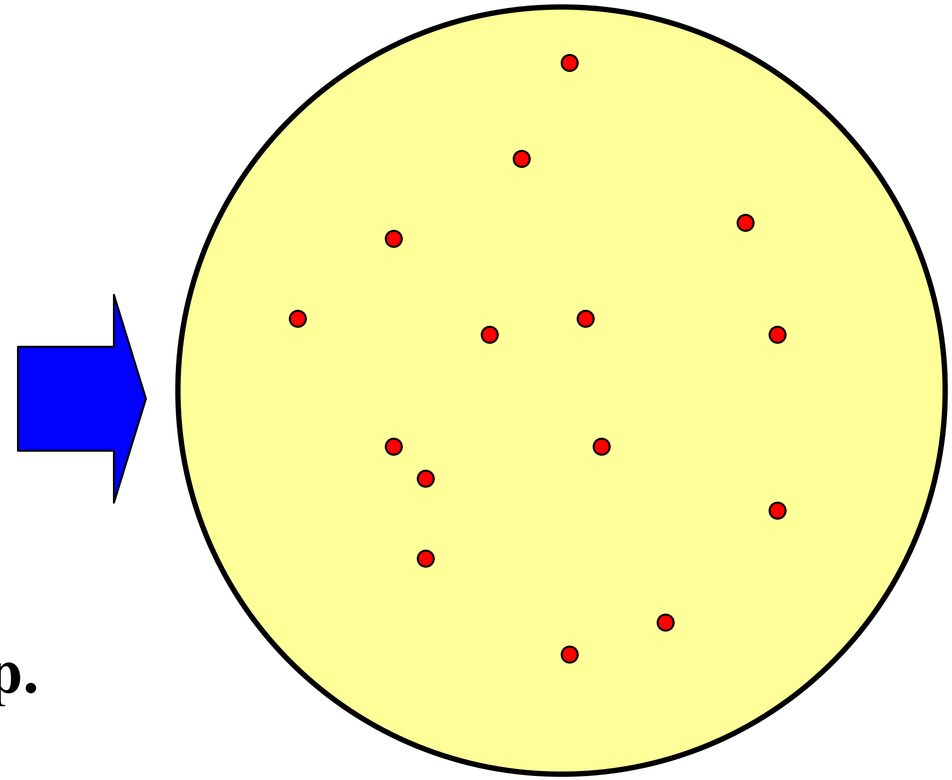
So, 20 colonies on 10^{-4} plate = 2×10^5 CFU/ml

Overlayer technique for cultivation of acidophilic Fe(II)-oxidizing bacteria (D.B. Johnson)

Problem: hydrolysis of solidifying agents (agar or agarose) inhibits growth of Fe(II) oxidizers

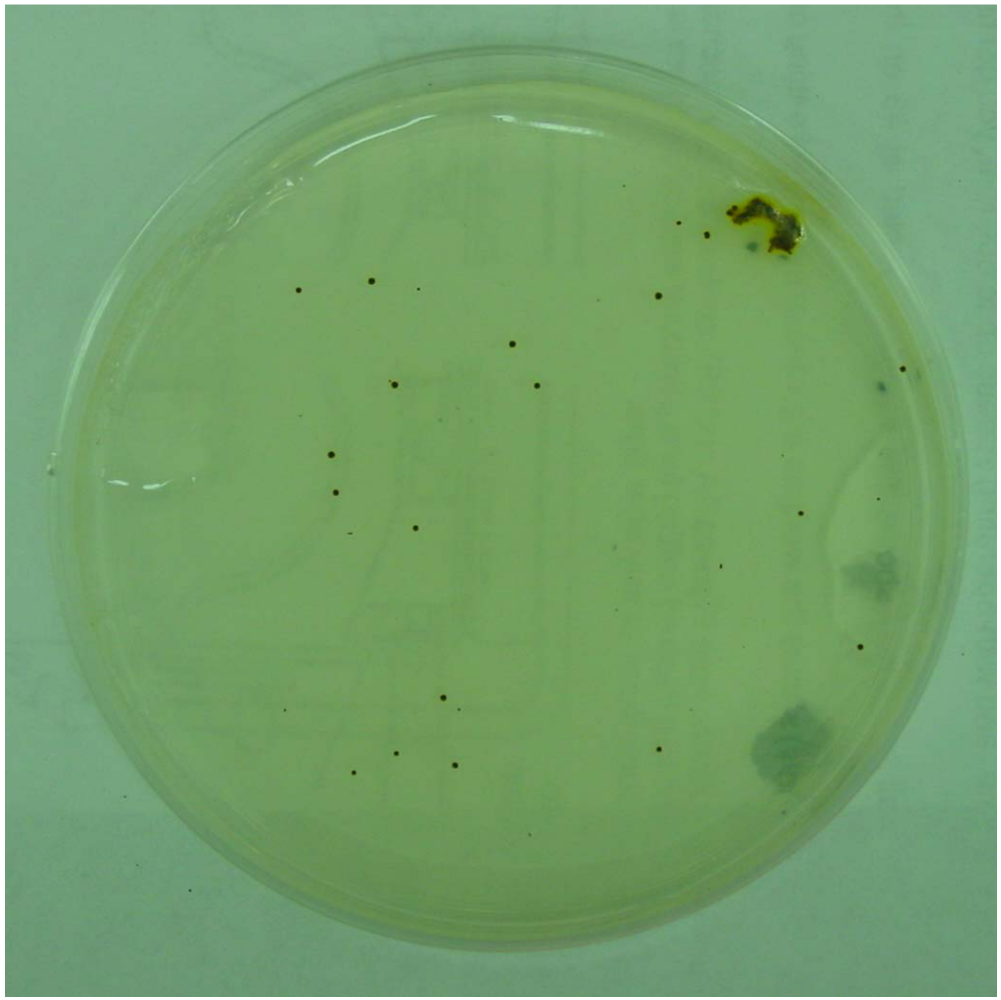
Environmental samples can be spread on heterotroph-free top layer; medium contains Fe(II) & nutrients, but no organic electron donor



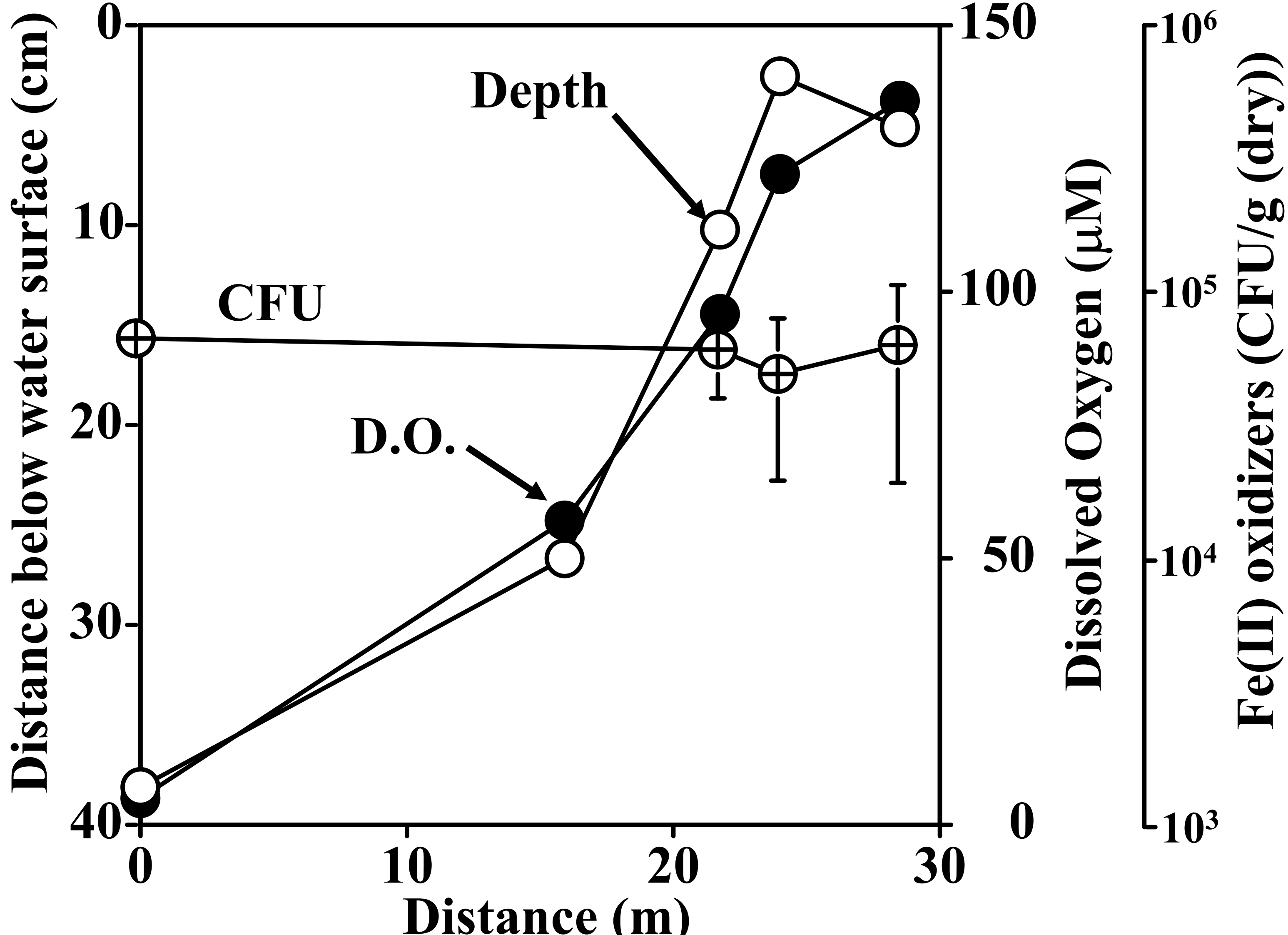


Inoculated with *Acidiphilium* sp.
- **acidophilic heterotroph**
- **consumes hyrolysis products**

Acidophilic Fe(II) oxidizers



Enumeration of aerobic Fe(II)-oxidizing bacteria in Glenwhite discharge

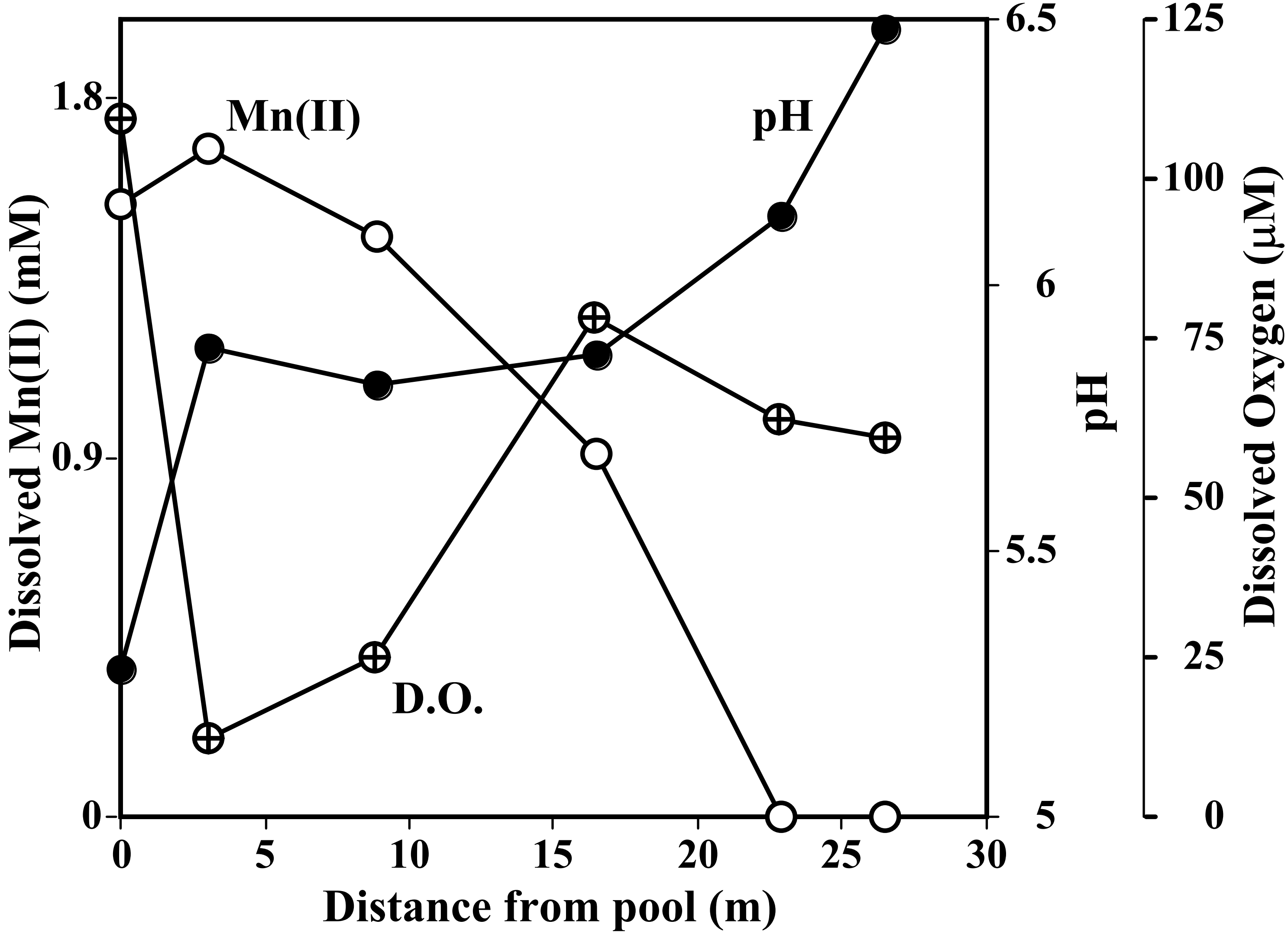


Glenwhite conclusions & future work

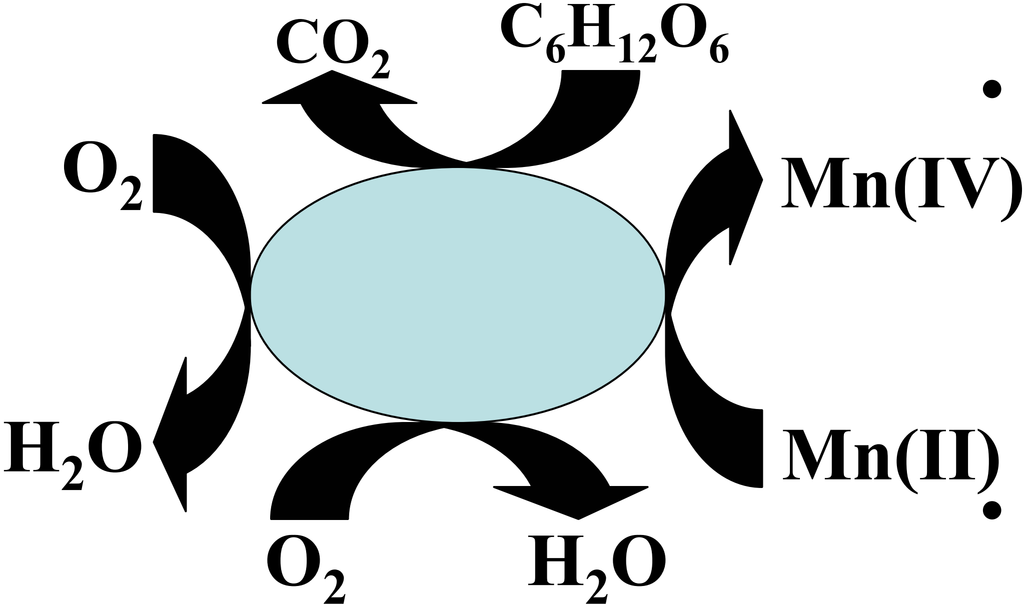
- Oxygen levels increase as water moves through Glenwhite discharge stream
- A denser, more crystalline Fe(III) precipitate forms in water containing less oxygen.
  - Presence of goethite in low pH water
- Culturable Fe(II) oxidizing bacteria present in similar numbers throughout discharge stream
- Future work:
  - examine microbial community structure using nucleic acid-based techniques
  - Determine the importance of microbiological activity vs. abiotic precipitation in Fe(III) precipitation at low pH
  - Examine the effect of oxygen on activity and Fe(III) products produced by acidophilic Fe(II) oxidizers

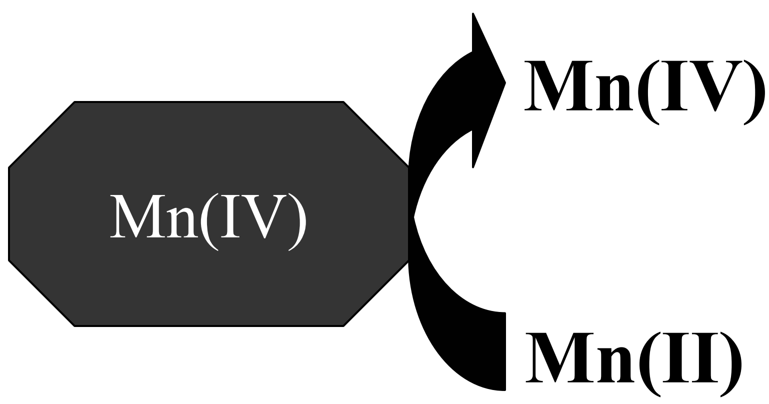
Fairview

Water chemistry in “ditches” in Mn removal beds, Fairview site



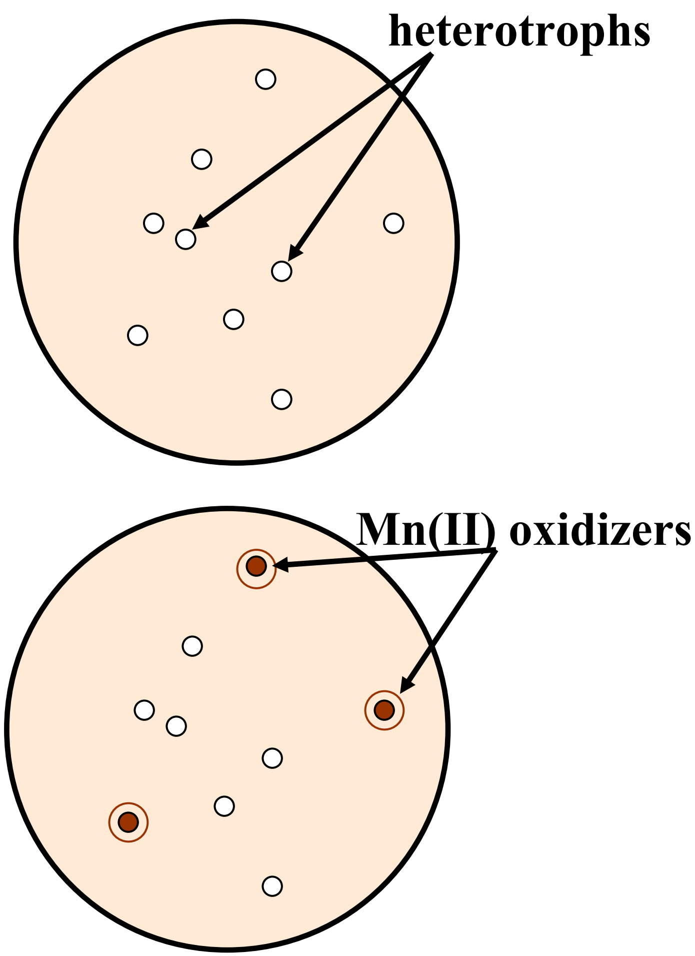
Biooxidation of Mn(II)





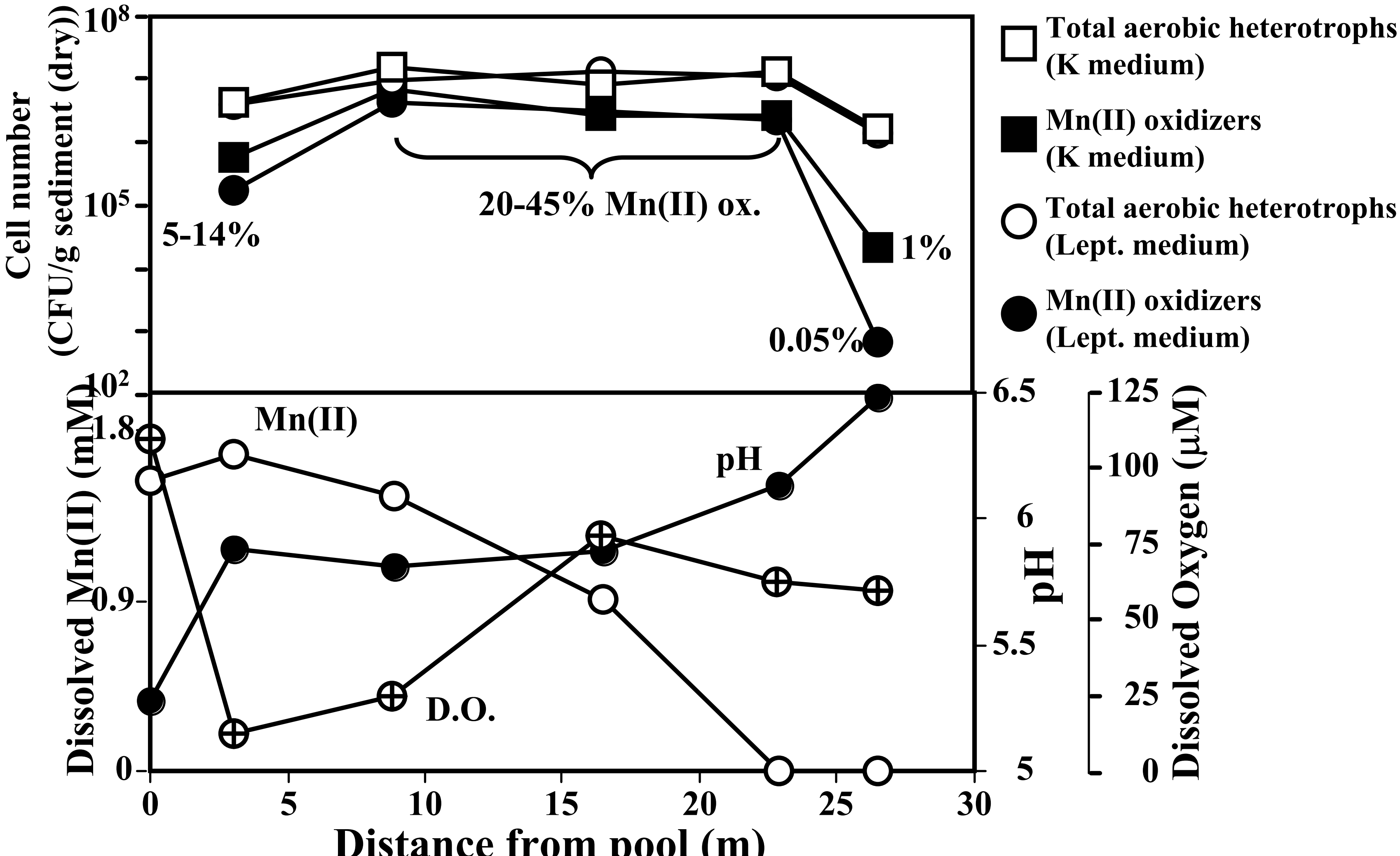
- **Energy from Mn(II) oxidation coupled to O_2 reduction not known to be exploited for growth (so far)**
- **Organoheterotrophic, neutrophilic**
- ***Leptothrix* sp., *Bacillus* sp., *Pseudomonas* sp.; relatives of *Rhodobacter* sp., *Agrobacterium* sp.**

Culture-dependent enumeration of Mn(II)-oxidizing bacteria



- Two types of media (Templeton et al., 2005):
  - K medium
  - Leptothrix medium
  - Both contained 100 mg/l cycloheximide (antifungal antibiotic)
  - Prepared using Fairview water

Enumeration of heterotrophic and Mn(II)-oxidizing aerobes in Fairview "ditches"

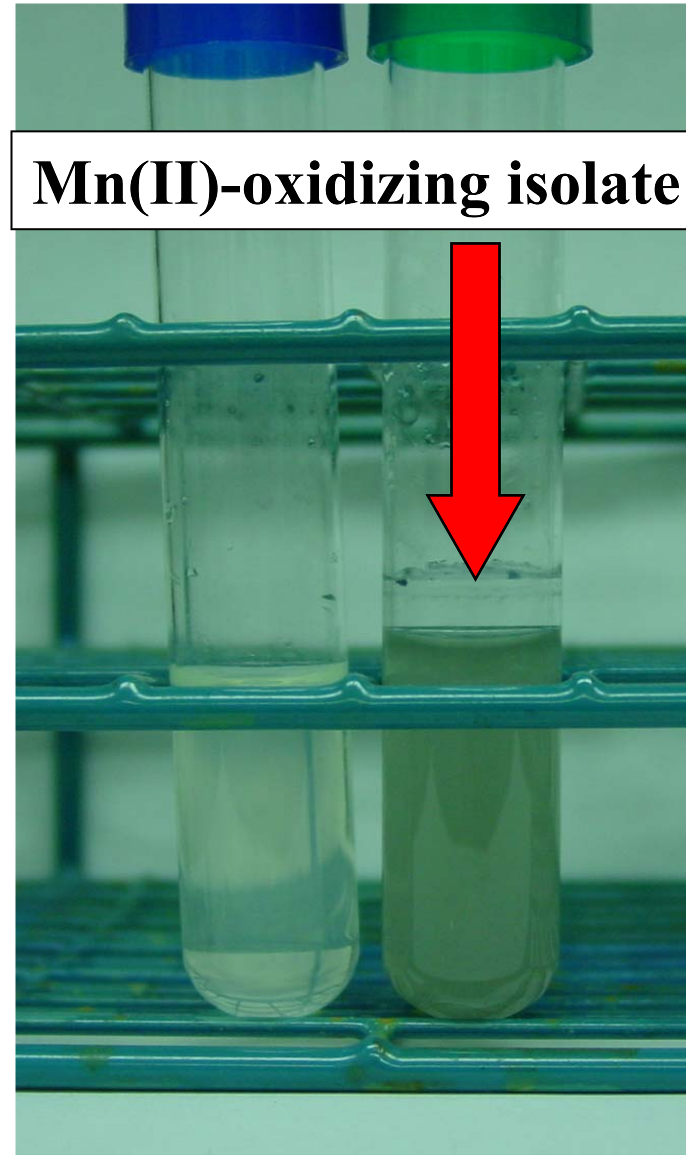


Fairview conclusions

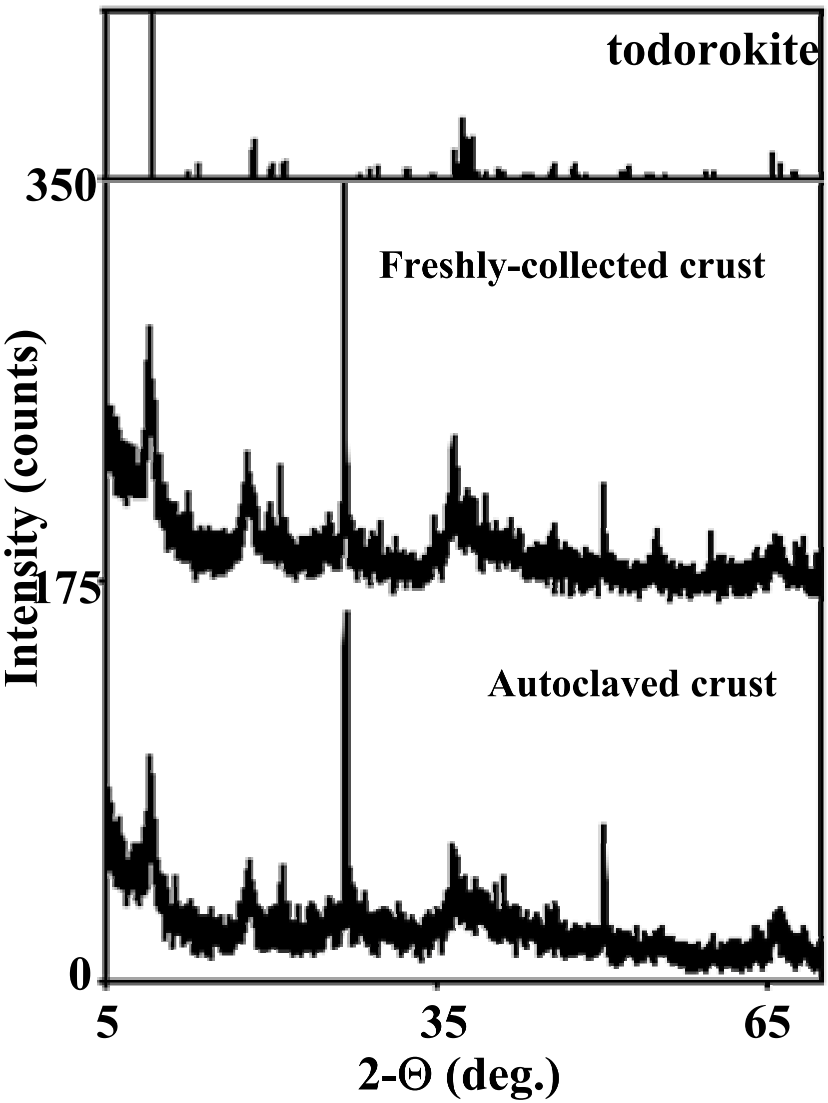
- **Mn removal beds effectively raise pH and remove Mn(II) (from 1.6 mM to < 0.05 mM)**
- **Mn(II)-oxidizing bacteria present in high numbers**
- **Little Mn(II) oxidation, low D.O., and lower numbers of Mn(II)-oxidizing bacteria in hay-filled trench**
- **"Paradox" of stimulating Mn(II) biooxidation:**
  - **Mn(II) oxidation takes place during late-log to stationary phase of growth (organic carbon problems?)**
  - **Need organic carbon to sustain sufficient biomass to catalyze Mn(II) oxidation at reasonable rates**

Future work

- **Establish the role of Mn(II)-oxidizing bacterial activity in Mn removal at this site**
  - **Differentiate between biological and abiotic activity**
  - **Sterile controls?**
- **Establish optimal conditions (e.g. D.O., organic substrate) for Mn(II)-oxidizing activity**



Characterization of Mn crusts from Fairview



	Freshly-collected crust	Autoclaved crust
Particle size (nm)	2601±1385	3587 ± 269
Zeta potential (mV)	-13.43 ± 0.18 (pH 6.98)	-13.15 ± 0.39 (pH 7.08)
HCl-extractable Mn (%) (Mn(II/III)	15.87	16.38
Hydroxylamine-extractable Mn (%) (Mn(IV))	84.13	83.62

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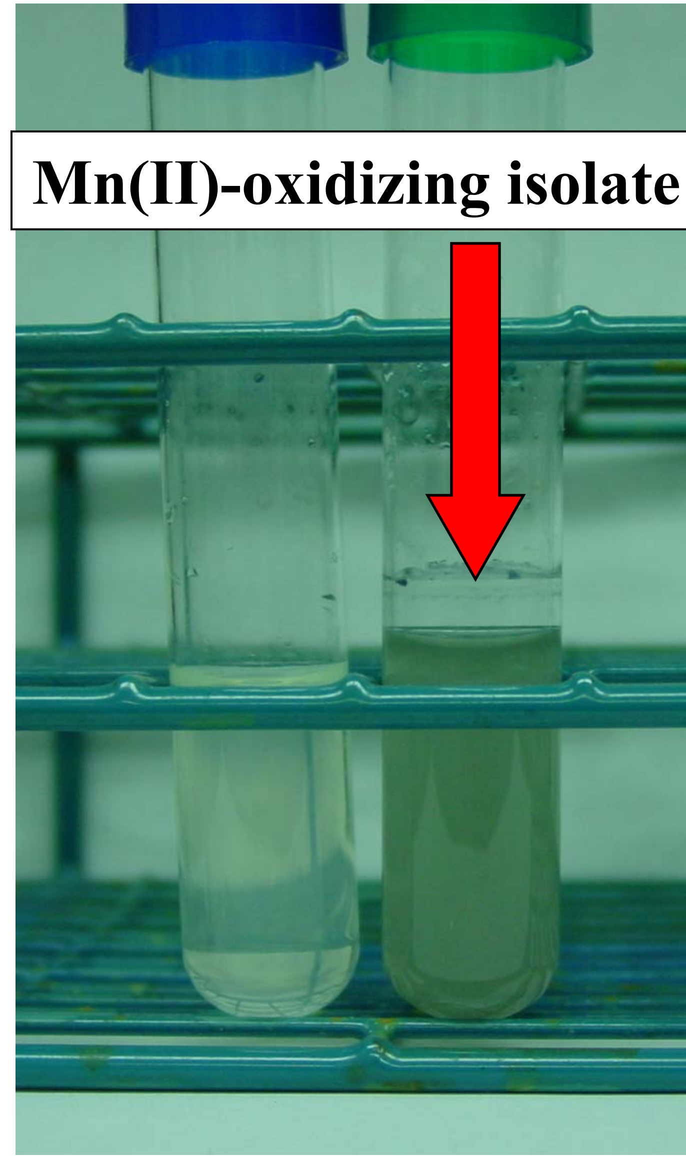
- Establish optimal conditions (e.g. D.O., organic substrate) for Mn(II)-oxidizing activity



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